**Fecal Sludge (Biosolids) Dilution Setup and MPN Calculation Protocol**
(NC WW/GW LC Policy 2/14/2020)

The following protocol, based on correspondence with US EPA Region IV and the Office of Research and Development in Ohio, must be followed when citing Standard Methods 9221 C E-2006 for the determination of the MPN Index/100 mL values that are geometrically averaged for use in the final MPN Fecal Coliform/gram calculation. This policy does not apply when citing EPA Methods 1680 or 1681. Those methods are to be followed as written.

The guidance provided in "Control of Pathogens and Vector Attraction in Sewage Sludge" (EPA/625/R-92/013), more commonly known as the White House document, is to be followed for setting up the series of sample dilutions. If the laboratory is unfamiliar with the samples, it is recommended that a series of five dilutions, instead of four, be set. Once the dilutions have been set, follow the procedure in Standard Methods 9221E-2006 for incubation and interpretation of gas production. Follow the procedure in Standard Methods 9221C-2006 for narrowing the series of dilutions down to three.

Use Table 9221:IV to determine the MPN Index/100 mL.

The geometric average of the individual results determined from the procedure above is then used in the following formula to calculate the final result for reporting.

$$\text{MPN Fecal Coliform/g} = \frac{10 \times \text{MPN Index/100 mL}}{\text{largest volume} \times \%\ \text{dry solids}}$$

NOTE: "% dry solids" is **not** in decimal form.

NOTE: "largest volume" refers to the volume of the original mixture (e.g., 30g in 270 mL) that is in 1 mL of the solution that is actually incubated.